Team iGEM UFSCar-Brasil

BATCH PURIFICATION OF ZINC PROTEINS FROM E. COLI UNDER NATIVE CONDITIONS

1. Take a right previously prepared Zn-NTA resin cartridge to a bench support.
2. Flow the storage solution (20% EtOH).
3. Wash the column with 5 volumes of destilled water.
4. Recharge the column with 2 volumes of 100 mM $ZnCl_2$ or $ZnSO_4$.
5. Wash the column with 10 volumes of destilled water.
6. Equilibrate with 5 volumes of a suitable buffer (e.g., Lysis buffer).
7. Flow the cleared cell lysate and save flow through.
8. Wash the column with 2 volumes of appropriate buffer.
9. Repeat the step 8.
10. Repeat the step 8.
11. Elute the proteins with 1 volume of elution buffer.
12. Repeat the step 11.
13. Repeat the step 11.
14. Set the samples in a SDS-PAGE with the following sequence of samples:

Marker, Cell lysate, Flow through, Wash 1, Wash 2, Wash 3, Elution 1, Elution 2,

Elution 3

BUFFERS AND SOLUTIONS RECIPES

Lysis buffer (1 liter):
50 mM NaH_2PO_4 6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)
10 mM imidazole 0.68 g imidazole (MW 68.08 g/mol)
Adjust pH to 8.0 using NaOH.

Wash buffer (1 liter):
50 mM NaH_2PO4 6.90 g $NaH_2PO_4 \cdot H2O$ (MW 137.99 g/mol)
300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)
20 mM imidazole 1.36 g imidazole (MW 68.08 g/mol)
Adjust pH to 8.0 using NaOH.

Elution buffer (1 liter):
50 mM NaH_2PO_4 6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)
250 mM imidazole 17.00 g imidazole (MW 68.08 g/mol)
Adjust pH to 8.0 using NaOH